Association Study of Single Nucleotide Polymorphisms in KDM3A and LOC203413 Genes with Male Infertility

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**Introduction**

Infertility is a worldwide problem affects 15% of the couples [1-3]. Male infertility refers to the inability of a male to achieve pregnancy in a fertile female. Among married couples, nearly 10% of them go childless despite unprotected intercourse and nearly 50% of this problem is solely due to the male partner [2,4,5]. But nearly in 60-75% male infertile cases, the underlying cause of infertility remains undefined which are referred to as idiopathic infertility [6,7]. A recent report in India, states that nearly 50% of infertility is related to the reproductive anomalies or disorders in the male [4]. Most of the cases are likely to be of genetic origin because spermatogenesis and fertility are controlled by large number of genes and the roles of very few genes are being explored [8,9]. Other than the Y chromosomal defects, the profound genetic cause of male infertility, to date only very modest information is known about non-Y-chromosomal gene defects including autosomal genes and the genes in X-chromosome. The non-Y-chromosomal genes are now gaining increased attention in their role in spermatogenesis and male fertility. Therefore the gene deletions or the SNPs in the genes involved in spermatogenesis process can be accounted for male infertility [5]. A pilot genome-wide SNP association study (GWAS) for male infertility and a follow-up study [10,11] identified numerous SNPs in many autosomal genes and in X chromosome genes. A number of reports have focused on the role of certain haplogroups, allelic variants and single nucleotide polymorphisms in male infertility [12].

The SNPs rs2030259 and rs5911500 in genes KDM3A (lysine (K)-specific demethylase 3A) and LOC203413. The SNPs were identified by restriction fragment length polymorphism (RFLP) technique in 17 normospermic individuals and in 13 infertile males (9 oligospermic and 4 azoospermic) and results were analysed using fisher’s exact test. Although the polymorphisms were found in both fertile and infertile men, no significant association is found for both 2 SNPs with infertility from the samples observed.

<table>
<thead>
<tr>
<th>SNP rs2030259 allele frequency</th>
<th>G</th>
<th>A</th>
<th>Total</th>
</tr>
</thead>
</table>

A case-control study conducted on fertile and infertile men in Coimbatore population. The study, aimed to identify and associate the following two single nucleotide polymorphisms (SNPs) rs2030259 and rs5911500 in genes KDM3A (lysine (K)-specific demethylase 3A) and LOC203413. The SNPs were identified by restriction fragment length polymorphism (RFLP) technique in 17 normospermic individuals and in 13 infertile males (9 oligospermic and 4 azoospermic) and results were analysed using fisher’s exact test. Although the polymorphisms were found in both fertile and infertile men, no significant association is found for both 2 SNPs with infertility from the samples observed.

Table 1: Contingency table for SNP rs2030259 in KDM3A gene. The two tailed p-value equals 0.4069.

**Materials and Methods**

**Study samples**

The study sample includes 17 fertile (control) men and 13 infertile men (9 oligospermic and 4 azoospermic) of Coimbatore district, Tamil Nadu. The study was performed as two phases (proposal no. 13/017, 16/151) and were approved by Institutional Human Ethics Committee of PSG Institute of medical sciences and research (IHEC, PSG IMS&R), Coimbatore.

**Inclusion and exclusion Criteria**

The sample size is 30. The inclusion criteria for fertile (control) men are age between 25-50 years having children. In case of infertile men, the inclusion criterion is age between 25-50 years.

**Blood sample collection**

After obtaining written consent from infertile patients and control men, 5 ml of blood drawn using plastic disposable syringe under aseptic conditions. The collected blood samples were stored in an EDTA/tri sodium citrate coated containers at 4°C.

**Genomic DNA isolation and quantification**

Genomic DNA was isolated from the collected blood samples using salting out method [14]. The extracted genomic DNA was quantified using Nanodrop spectrophotometer and all the samples were diluted
with TE buffer to the final concentration of 50 ng/μl, which was used for all PCR amplification.

PCR amplification and restriction analysis

The SNP region of respective genes was retrieved from NCBI SNP database and the primers for the SNP region were designed using Primer3 Input (version 4.0.0) tool. The primers 5'-GGACACGGTGAGGTGCTCTGA-3' and 5'-GAACGGAGAAAAGGAGGAAG-3' (Integrated DNA Technologies) were used to amplify the SNP rs2030259 in KDM3A gene and the respective restriction enzymes, AanI (PsiI) (ER2061, Fermentas, Thermo Scientific) for the SNP rs2030259 and TaqI (FD0674, Fermentas FastDigest™, Thermo Scientific) for the SNP rs5911500 were used to perform restriction analysis according to the enzyme manufactures protocol.

In short, the restriction mixture contains 10 μl (0.1-0.5 μg of DNA) of PCR product, 18 μl nuclease free water, 2 μl 10X restriction buffer, 1-2 μl specific restriction enzyme and the mixture were gently spin down for few seconds and incubated at 37˚C (AanI) and 65˚C (TaqI) for 1 hour. Then the restricted products were resolved in 2% agarose gel at 70 V.

Results

For all the samples (17 fertile and 13 infertile), genomic DNA were isolated and both the SNP regions were amplified using specific primer sets.

Following the PCR amplification of the SNP regions, restriction analysis was performed and the restricted products were resolved using 2% agarose gel electrophoresis and visualized by gel documentation system.

The restriction digested samples for both SNPs resolved in agarose gel and the documented image follows. The figure 1, 3 shows that among the 17 fertile samples, eight samples (Figure 1, in lane 3, 12, 13 and Figure 3, in lane 2,3,4,5,6 ) were wild type (G/G), 4 samples (Figure 1,in lane 5, 6, 9, 10) were homozygous (A/A) mutants and 5 were heterozygous (G/A) mutants (Figure 1, samples in lane 2, 4, 7, 8, and Figure 3, in lane 2,3,4,5,6 ) were wild type (C) and the one (sample in lane 9) was mutant (T). Figure 7 shows 4 mutant types (lane 2,3,4,5) and 1 wild type (lane 6) for the polymorphism (C/T). In case of patient samples for the SNP rs5911500 the figure 6 shows that among the 8 patient samples, seven (samples in lane 2-8) were wild type (C) and the one (sample in lane 9) was mutant (T). Figure 8, shows, 3 wild type (lane 5, 7, 8) and 2 mutant types (lane 4, 6) for the polymorphism (C/T) (Table 2).

Table 2: Contingency table for SNP rs5911500 in LOC203413 gene.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (fertile)</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>case (infertile)</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>4</td>
</tr>
</tbody>
</table>

Statistical analysis

The study compares the differences in frequencies of an allele between infertile and fertile population, either fisher’s exact test or X² (chi square) test can be performed. Since the sample size is small (30), fisher’s exact test was performed to evaluate the statistical significance of the SNPs with infertility.

Discussion

Although the causes for infertility were widely studied, the etiology across different populations still varies and remains poorly understood. In the current study, autosomal and X-chromosome SNPs rs2030259 and rs5911500 SNPs associated with infertility were...
evaluated for the association with male infertility. This study found no significant association of SNPs with infertility, which is in contradiction with results of Aston et al. and Plaseski et al. [4,16]. No greater difference was found in the allelic frequency between the fertile and infertile group and it may due to uneven sample size (Table 3).

<table>
<thead>
<tr>
<th>Sample size</th>
<th>rs2030259</th>
<th>rs5911500</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G/G (WT)</td>
<td>A/A (HG)</td>
</tr>
<tr>
<td>Fertile (12)</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Infertile (8)</td>
<td>9</td>
<td>1</td>
</tr>
</tbody>
</table>

WT-Wild type, HG-Homozygous, HT-heterozygous, MT-mutant

Table 3: Prevalence of SNPs in fertile and infertile samples.

This disagreement may be due to difference in the population studied or the SNPs were population specific and due to smaller and uneven sample size. As there is no significant association of SNPs with infertility, the other possible explanation for this discrepancy is that these SNPs may not be responsible for the infertility, but more likely its linkage with other SNPs in the same genes or in other genes might have an effect on spermatogenesis and it is to be evaluated. The two tailed P-Value equals 1. The p-values should be less than 0.05 to be statistically significant. Since we got the P values higher than 0.05, there is no statistically significant association between the SNPs and male infertility. From many association studies it can be speculated that male infertility is similar in nature to the majority of complex diseases studied to date, that the disease have multigenic cause and no single SNP is responsible for an appreciable proportion of disease [11]. Hindorff et al. [12] identified genetic variants associated with different diseases through genome-wide studies and it has been understood that thousands of cases and controls will be required to capture an candidate genes and their abnormalities that may play a role in these SNPs may not be responsible for the infertility, but more likely its contradiction with results of Aston et al. and Plaseski et al. [4,16]. No infertility. Also such studies with larger independent samples of control studied or the SNPs were population could be useful to populations study has to be enriched with more number of samples with wide.

Limitations

Family studies could not be performed.

References